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CHANGES IN THE STRUCTURE OF THE PEEP VOCALIZATIONS OF FEMALE DOMESTIC CHICKS (*GALLUS GALLUS DOMESTICUS*) WITH AGE

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ABSTRACT: Changes in the structure of the peep vocalization of female domestic chicks reared in pairs from day three post-hatching were investigated. Recording began on day five with each chick being recorded in isolation twice weekly over a ten week period post-hatching. Spectrographic analysis shows that the peep call develops an increasingly complex structure from the second week post-hatching with additional components introduced as the chicks age. Nine acoustic parameters (duration, maximum frequency, minimum frequency, difference between maximum and minimum frequency, peak frequency, peak frequency range, peak amplitude, energy and average power) of four different peep calls were analysed. Significant differences were found between the four types of peep call on seven of the nine acoustic parameters. Discriminant analysis showed that the different types of peep call could be accurately determined on the basis of these results. Correlations of the call parameters showed that the calls displayed lower levels of stability as call structure became more complex. Chicks also displayed high inter-individual variation and relatively low intra-individual variation over call parameters. Results are discussed in relation to hen-chick, chick-chick communication as the broody period declines.

The chick of the domestic fowl vocalizes before hatching (Guyomarc'h, 1974) and from as early as day 18 of incubation (Gottlieb and Vanderbergh, 1968). Tuculescu and Griswold (1983) classified these pre-natal calls as distress calls and pleasure calls. Greenlees (1993) showed spectrograms of these pre-hatching calls and concluded

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these calls were similar in structure to the post-hatching calls of the chick. Post-hatching the peep and twitter constitute the main call types (Kaufman and Hinde, 1961; Montevecchi, Gallup and Dunlap, 1973). Studies into causation show that the peep call is emitted in a variety of situations, such as a change in environmental temperature (Kaufman and Hinde, 1961), maternal isolation (Bermant, 1963) and removal of an imprinting object (Brown, 1979).

Spectrographic analysis has shown distinct structural differences between peeps and twitters (Collias, 1952; Collias and Joos, 1953; Guyomarc'h, 1962; Andrew, 1963, 1964; Collias, 1987). Peeps are characterised by descending frequencies while twitters tend to swing upwards in pitch (Collias, 1987). The 'distress call' (Collias and Joos, 1953), ÔLe cri d'appel du Poussin isol Õ (Guyomarc'h, 1962), and 'peeps' (Andrew, 1964) have all been classified by Wood-Gush (1971) as being the same call. Jennings and Kent (1996) demonstrated that three types of peep call can be produced by the same chick in the first week of life. These three types of peep had descending frequencies but with differing acoustic parameters.

Schjelderup-Ebbe (1913) described a number of vocalizations emitted by male and female fowl and described them in relation to social behaviour. According to Konishi (1963) the most complete verbal description of the vocal repertoire was conducted by Baeumer (1962). Spectrograms illustrate the range and complexity of fowl vocalizations (Collias, 1952; Collias and Joos, 1953; Konishi, 1963). Collias (1987) attempted the first spectrographic analysis and classification of the complete repertoire in a species of fowl *(Gallus gallus)*. However, Collias (1987) restricted his analysis to only chick and adult calls. Brückner (1933) pointed out that the structural and vocal traits of chicks continually change with age.

Vocal development has been extensively studied in songbirds (e.g. Nelson et al., 1997). In contrast, call development in non-songbird species has received less attention (Ballintijn and ten Cate, 1997). However, recent research has shown that in another non-songbird species, the collared dove (*Streptopelia decaocto*; Ballintijn and ten Cate, 1997), vocal changes gradually occur with age. These studies indicate that whether through social interactions (for review see Baptista and Gaunt, 1994) or through some innate mechanism (Konishi, 1963; Nottebohm and Nottebohm, 1971), changes do occur in the acoustic and structural properties of juvenile vocalizations as they age. The syrinx is the main vocal organ in birds (Brackenbury, 1982) and as such, changes in vocalizations are likely to be related to changes in

syringeal anatomy (Ballintijn and ten Cate, 1997). In addition, Podos (1996) has indicated that motor constraints also play a role in vocal performance in swamp sparrows. It is proposed here to redress the current research bias in favour of songbird species by regular recording of a vocalization of domestic chicks as they age to discover if and how their calls develop. Accordingly, the purpose of this paper is to describe changes in structure of the peep call of female domestic chicks as they age from hatching to ten weeks post-hatching.

METHOD

Subjects

Sixteen brown leghorn chicks from two batches of eggs were randomly placed in pairs and reared together from day three post-hatching. Chicks were incubator hatched from eggs produced by the same flock of hens. The first batch (Batch A) contained four chicks, (1 male and 3 females) and Batch B contained twelve chicks, (3 males and 9 females). No pen contained more than one male. Male chicks were eliminated from the statistical analysis because of the small sample size, thus 12 female chicks were used.

Procedure

The procedures used in this paper are the same as those described in Jennings and Kent (1996). In addition, at four weeks the infrared heat lamps were removed as the chicks had enough plumage to enable them to stay warm, and the batch B testing procedure was used with all chicks. During recording an observer was situated behind a wooden screen with a 5 cm x 26 cm slot which allowed observation of the chicks with minimal interference. A tape recorder, located on a table behind the observation screen, was operated by the observer.

Measures

Twelve chicks emitted the long peep (week 1 post-hatching) and two component peep (weeks 2-4 post-hatching), eleven chicks emitted the three component peep (weeks 4-6 post-hatching) and component under peep (weeks 6-10 post-hatching). The chicks' vocalizations were analyzed using Canary 1.1 with a filter bandwidth set at 352.94 Hz on a

Macintosh II vx. Data were collected by selecting an entire call, pressing the command key and clicking the mouse button. This transferred the data to the Canary data log. The parameters chosen for analysis were:

1. Duration (ms)
2. Maximum Frequency (kHz)
3. Minimum Frequency (kHz)
4. Difference between Max. and Min. Frequency (kHz)
5. Peak Frequency (kHz)
6. Peak frequency range (kHz)
7. Average Power (watts)
8. Energy (joules)
9. Peak Amplitude (volts)

Duration refers to the length of the call. Maximum frequency and minimum frequency refer to the highest and lowest points respectively as assessed by the call spectrogram. Calls were enlarged to the size of the monitor to ensure accuracy. Peak frequency refers to the frequency with the highest amplitude. Peak frequency range was computed as the peak frequency minus the minimum frequency; this parameter gives the distance (in kHz) between peak frequency and minimum frequency. Parameters [7-8] give details of energy use. Analysis of the nine acoustic parameters was completed using the Statistical Package for the Social Sciences version 6.1 for Windows (SPSS 6.1; Nie *et al.*, 1975). Three examples of each call type were randomly selected and then analysed.

Statistical Procedures

The data for each call type for each chick were averaged and analysed using analysis of variance (ANOVA). Post hoc comparisons were conducted using Duncan's multiple range test, which allows a sequential comparison of the four groups of peep calls (Ferguson and Takane, 1989). Due to software constraints it was not possible to capture highest frequencies of some of the peep calls, therefore, a nominal maximum frequency of 11.1 kHz was assigned for peep calls when the maximum frequency was not available. In order to assess the level of variation across parameters, a discriminant function analysis was computed on variables that provided significant results in ANOVA. Pearson product moment correlations were computed between calls of the same type in order to assess the level of call stability that each vocalization type exhibited. The possibility that individual variation in

the peeps of chicks increased as they aged was examined using Levene's test for homogeneity of variances.

RESULTS

A description of the four peep calls

Variations in the peep call were studied over 10 weeks post-hatching. Calls were identified first by an aural inspection of the cassette tapes and then by a visual inspection of the spectrograms. Similar methods of call selection have been used by Seyfarth, Cheney and Marler (1980) and Jennings and Kent (1996). Four different types of peep call (Figure 1) were included in the analysis. These calls were all recorded at different ages. The long peep was recorded in the first week post-hatching (Figure 1 (a)) and described in detail previously (Jennings and Kent, 1996). The long peep has a complex structure with a wide frequency range. There is an upper inversion located at the top of the descending limb of the peep at 5 kHz. The two component peep, recorded from weeks 2 to week 4 post-hatching (Figure 1 (b)), has a wide frequency range similar to the long peep. This call also has an upper inversion at the top of the descending limb located at about 4 kHz. There is a second component located above the fundamental at 8 kHz which runs parallel to the limb at 4 kHz. The three component peep, recorded on weeks 4 to 6 post-hatching (Figure 1 (c)), is similar in structure to the long peep and two component peep. This peep call displays three components within its structure: the first at 3 kHz, the second located at 6 kHz and the third at 8 kHz. This call displays the typical structure of a peep call, with all three components descending in frequency over the duration of the call. The component under peep was recorded on weeks 6 to 10 post-hatching (Figure 1 (d)) and has several component bands located in the call. This call does not display any structure at the top of the descending limb. The distinguishing feature is the presence of component structure below the main descending limb at 1 kHz. Figure 1 shows that as the chicks age the structure of the peep calls becomes increasingly more complex.

Changes in the structure of the peep call were associated with an increase in the age of the chicks. However, the parameters of maximum frequency, minimum frequency and difference between max. and min. frequencies showed little variation between the peep calls (Table 1). There is an increase in the range and standard deviation

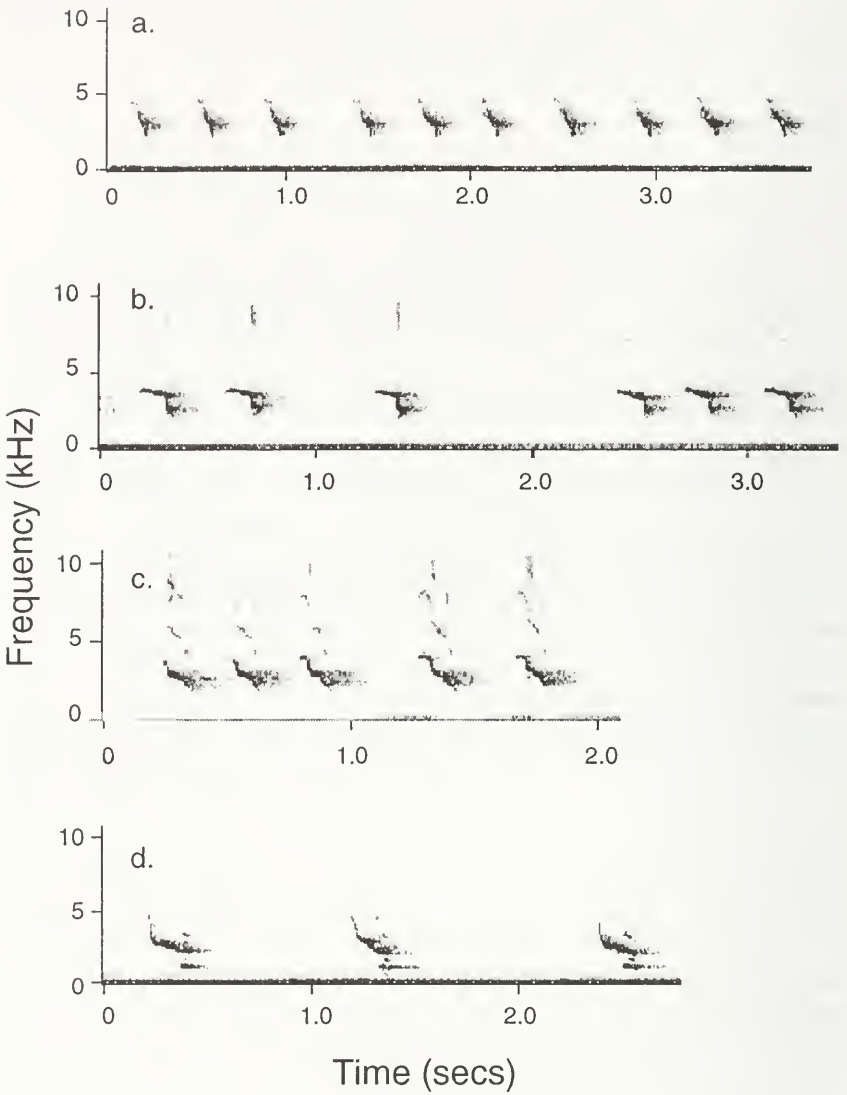

Figure 1. Spectrograms of the four call types analyzed: a, the long peep; b, two component peep; c, three component peep; and d, component under peep.

between the peep calls for these parameters. The mean of average power displays an increase from the long peep to the two component peep which then falls as the number of components increases. Standard deviation for the different parameters increases over call types, showing variation in calls as the chicks aged. The mean for energy and peak amplitude increases as the calls become structurally more complex;

there is also an increase in the standard deviations shown by calls over these two parameters. The mean for the parameter of peak frequency decreases as the chicks age and the standard deviation increases.

Table 1. Mean, range, standard deviation (S.D.) and coefficient of variation (C.V.) for the four calls at nine different parameters.

Parameters	Call Types	n	Mean	Range	S.D.	C.V.
Duration	Long Peep	12	329.8	112.7	36.8	11.16
	Two Component	12	331.7	105.7	29.4	08.86
	Three Component	11	361.9	226	66.1	18.27
	Component under	11	402.3	191	49.3	12.26
Max. frequency	Long Peep	12	11.1	0.1	0.039	0.35
	Two Component	12	11.0	1.15	0.331	3.00
	Three Component	11	10.7	3.067	0.934	8.73
	Component under	11	10.5	2.967	1.018	9.70
Min. frequency	Long Peep	12	1.525	2.033	0.787	51.61
	Two Component	12	1.181	2.167	0.860	72.82
	Three Component	11	1.627	1.000	0.321	19.73
	Component under	11	0.944	0.433	0.129	13.67
Diff. between max. and min. frequency	Long Peep	12	9.5	2.133	0.794	08.36
	Two Component	12	9.9	2.200	0.821	08.29
	Three Component	11	9.1	3.967	1.079	11.86
	Component under	11	9.5	3.167	1.047	11.02
Peak frequency	Long Peep	12	3.564	0.967	0.258	07.24
	Two Component	12	3.323	1.433	0.461	13.87
	Three Component	11	2.779	1.267	0.42	15.11
	Component under	11	2.448	1.833	0.506	20.67
Peak frequency range	Long Peep	12	2.042	3.0	0.861	42.17
	Two Component	12	1.944	4.0	0.996	51.24
	Three Component	11	1.056	2.8	0.640	60.61
	Component under	11	1.281	2.2	0.757	59.10
Average power	Long Peep	12	209.6	177.4	51.508	24.57
	Two Component	12	291.0	221.7	67.907	23.34
	Three Component	11	263.3	214.4	74.847	28.43
	Component under	11	223.9	258.8	85.55	36.58
Energy	Long Peep	12	29.5	28.53	8.034	27.23
	Two Component	12	37.3	29.47	7.616	20.42
	Three Component	11	47.2	63.87	16.196	34.31
	Component under	11	47.2	47.87	17.259	36.57
Peak amplitude	Long Peep	12	77.7	7.733	2.144	2.76
	Two Component	12	77.4	3.267	1.025	1.32
	Three Component	11	77.9	5.167	1.658	2.13
	Component under	11	78.7	6.600	2.045	2.60

A one way analysis of variance (ANOVA) on the nine parameters investigated differences between the calls. Significant differences between the four peep types were found for seven of the nine parameters (duration, min. frequency, peak frequency, peak frequency range, average power, energy and peak amplitude; Table 2).

Table 2. ANOVA for the nine parameters on four types of peep vocalization. Total Number of calls was 45 and the degrees of freedom were 3,42.

Parameters	Calls	
	F	P
Duration (ms)	5.9334	0.0018
Max. Frequency (kHz)	1.5801	NS
Min. Frequency (kHz)	3.3580	0.0275
Diff. btw max. and min. freq. (kHz)	1.1606	NS
Peak Frequency (kHz)	15.8153	0.0001
Peak frequency range (kHz)	3.7429	0.0180
Average Power (joules)	3.2490	0.0311
Energy (watts)	4.9582	0.0049
Peak Amplitude (volts)	13.1084	0.0001

Multiple comparisons using Duncan's method revealed significant differences between the component under peep and the other three types of peep call for the parameters of duration (ms) and peak amplitude (volts). Significant differences were found between the component under peep and the long peep, three component peep for minimum frequency (kHz). Significant differences were found between the long peep and the three component peep, component under peep for energy (watts). For average power (joules) a significant difference was found between the two component peep and the long peep, component under peep. Significant differences were also found between the long peep, the three component peep, component under peep; the two component peep and the three component peep, component under peep for the parameter of peak frequency (kHz; Table 3). A significant difference was found for the peak frequency range (kHz) between the three component peep and the long peep, two component peep.

A stepwise discriminant function analysis was conducted using the seven parameters that showed significant results in the ANOVA and post hoc multiple comparison procedures. This analysis was employed in order to assess the degree to which each acoustic parameter could be

Table 3. Multiple comparisons using Duncan's multiple range test. Comp., component; *, denotes significant differences between Call Type A and Call Type B at the .05 level; **, denotes differences at the .01 level.

Parameter	Call Type A	Mean Difference	Call Type B	Mean From
Duration (ms)	Long Peep	329.92**	Comp. Under Peep	402.27
	Two Comp. Peep	331.67**		
	Three Comp. Peep	362.00*		
Min. Frequency (kHz)	Long Peep	1.083*	Comp. Under Peep	0.367
	Three Comp. Peep	1.091*		
Peak Frequency (kHz)	Three Comp. Peep	2.778**	Long Peep	3.563
	Comp. Under Peep	2.449**		
	Three Comp. Peep	2.778*	Two Comp. Peep	3.233
	Comp. Under Peep	2.449**		
Peak Frequency Range (kHz)	Long Peep	2.042*	Three Comp. Peep	1.152
	Two Comp. Peep	2.056*		
Average Power (joules)	Long Peep	209.17*	Two Comp. Peep	290.42
	Comp. Under Peep	223.46*		
Energy (watts)	Three Comp. Peep	46.818**	Long Peep	29.583
	Comp. Under Peep	46.909**		
Peak Amplitude (volts)	Long Peep	77.167**	Comp. Under Peep	81.727
	Two Comp. Peep	76.833**		
	Three Comp. Peep	77.455**		

used to discriminate between the four call types. Results of the analysis produced three significant functions which distinguished between the four call types. Of the seven variables entered, peak frequency and duration provided the largest correlation in the first discriminant function. Energy and average power provided the largest correlation with the second function. The remaining three parameters were entered into the third function (Table 4). Results from Table 4 indicate that peak frequency and duration are the most important discriminating variables, although all seven variables that were entered contributed significantly to discrimination.

The degree of similarity between the four call types was assessed visually using a scatter plot (Figure 2) of the first two discriminant functions (peak frequency; duration/average power; energy). This

Table 4. Stepwise discriminant analysis showing the three significant functions computed and related statistics. * indicates largest absolute correlation between each variable and each discriminant function.

Parameter	Function 1	Function 2	Function 3
Duration (ms)	-0.12875 *	0.4227	-0.03281
Min. Frequency (kHz)	0.10453	-0.04222	0.18046 *
Peak Frequency (kHz)	0.20709 *	0.4227	-0.03281
Peak frequency range (kHz)	0.0719	0.08462	0.38014 *
Average Power (joules)	0.01339	-0.23472 *	0.15587
Energy (watts)	-0.10456	-0.1331 *	-0.12752
Peak Amplitude (volts)	-0.1743	0.19121	0.21327 *
Eigenvalue	25.1101	3.7936	0.7639
% Variance	84.64	12.79	2.57
Canonical Correlation	0.9807	0.8896	0.6581
Wilks' Lambda (λ)	0.00453	0.11827	0.566939
χ^2	215.884	85.391	22.7
df	18	10	4
Significance	0	0	0.0001

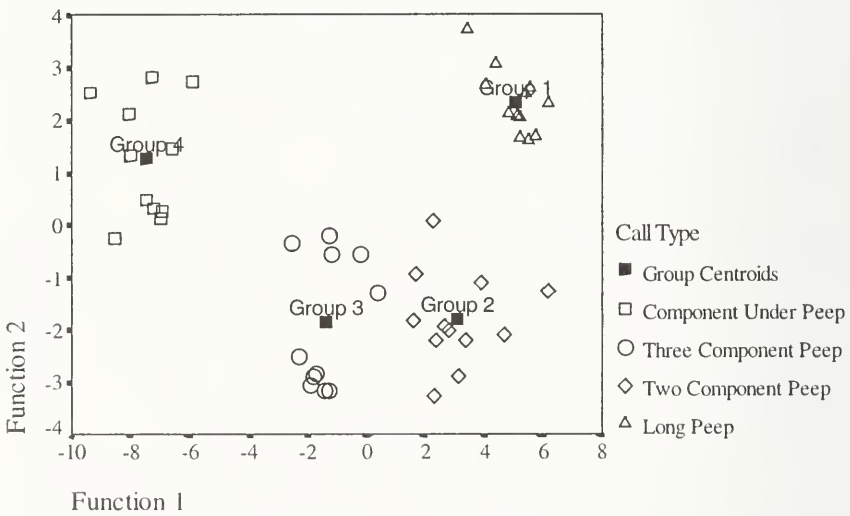

Figure 2. Scatterplot showing the separation of the four different types of peep call produced by the first two discriminant functions.

shows that each of the individual chicks clusters around each of the centroids for call type with no evidence of overlap among the four call types. In addition, the classification success of the three significant functions was 100%. This indicates that sufficient variation is exhibited by the call parameters included in the analysis to facilitate accurate prediction of call identity and to a lesser extent the individual identities of the chicks as they aged.

The stability of the Acoustic Parameters

The stability of the acoustic parameters was examined for the four call types using Pearson Product-moment correlations (r) (Table 5). For the long peep and two component peeps there are significant correlations between calls on all parameters at the .05 or .01 level. As the chicks aged calls became less stable as indicated by the lower correlations. The three component call shows significant correlations for eight of the nine parameters with one non-significant correlation for the parameter of energy. The component under peep shows two parameters with non-significant correlations (energy and low frequency). Furthermore, the correlations are increasingly showing .05 level significance.

Assessment of inter- and intra-individual variation in the four call types

The level of inter- and intra-individual variation exhibited by the calls was assessed over the nine acoustic parameters using Levene's test for homogeneity of variances. The results showed that there is considerable variation between the chicks for the four types of peep call (table 6). The call type that displays the highest variance between chicks (over parameters) is the component under peep followed by two component peep and the long peep. Intra-individual variance was also assessed using Levene's test. Results indicate that intra-individual variation in the call types is low with the exception of the component under peep which shows significant variance in five of the acoustic parameters (table 6).

Table 5. Pearson product moment correlations (r), three calls from each of the four call types were used in an examination of nine parameters.

Parameters	Call Type	n	r	P	r	P
Duration (ms)	Long Peep	12	.8258	.01	.8263	.01
	Two component	12	.8277	.01	.4906	.05
	Three component	11	.8375	.01	.7721	.01
	Component under	11	.5913	.05	.8733	.01
Maximum Frequency (kHz)	Long Peep	12	.6742	.05	.9393	.01
	Two component	12	.9978	.01	1.000	.01
	Three component	11	.6856	.05	.6768	.01
	Component under	11	.7246	.05	.9645	.01
Minimum Frequency (kHz)	Long Peep	12	.9927	.01	.9958	.01
	Two component	12	.9746	.01	.9803	.01
	Three component	11	.9219	.01	.9127	.01
	Component under	11	.1339	NS	.3062	NS
Diff btw max. and min. Freq. (kHz)	Long Peep	12	.9954	.01	.9946	.01
	Two component	12	.9886	.01	.9684	.01
	Three component	11	.7618	.01	.7126	.05
	Component under	11	.7421	.01	.9570	.01
Peak Frequency (kHz)	Long Peep	12	.9031	.01	.8949	.01
	Two component	12	.9978	.01	1.000	.01
	Three component	11	.8560	.01	.9563	.01
	Component under	11	.7008	.05	.9486	.01
Peak frequency range (kHz)	Long Peep	12	.9867	.01	.9858	.01
	Two component	12	.8084	.01	.7513	.01
	Three component	11	.8581	.01	.9311	.01
	Component under	11	.5956	.05	.8295	.01
Average Power (joules)	Long Peep	12	.9045	.01	.9348	.01
	Two component	12	.6682	.05	.7492	.01
	Three component	11	.7727	.01	.6535	.05
	Component under	11	.8357	.01	.7070	.05
Energy (watts)	Long Peep	12	.9128	.01	.9595	.01
	Two component	12	.7629	.01	.7035	.01
	Three component	11	.7872	.01	.5640	NS
	Component under	11	.5486	NS	.7559	.05
Peak Amplitude (volts)	Long Peep	12	.923	.01	.9408	.01
	Two component	12	.7300	.01	.8429	.01
	Three component	11	.8115	.01	.8408	.01
	Component under	11	.6914	.05	.7407	.05

Table 6: Levene's test for homogeneity of variances showing inter- and intra-individual variances for each acoustic parameter for each call type.

Call Type	Parameters	Inter-individual variances	Intra-individual variances
Long Peep	Duration	NS	NS
	Max. frequency	.0020	NS
	Min. frequency	.0350	NS
	Max. - Min. frequency	.0001	NS
	Peak frequency	.0001	NS
	Peak frequency range	.0030	NS
	Average Power	.0280	NS
	Energy	.0001	NS
	Peak Amplitude	.0030	NS
Two Component Peep	Duration	NS	NS
	Max. frequency	.0001	.016
	Min. frequency	.0001	NS
	Max. - Min. frequency	.0001	.021
	Peak frequency	.0001	NS
	Peak frequency range	.0001	NS
	Average Power	.0001	NS
	Energy	.0001	.019
	Peak Amplitude	.0001	NS
Three Component Peep	Duration	.0030	NS
	Max. frequency	.0001	NS
	Min. frequency	NS	NS
	Max. - Min. frequency	.0001	NS
	Peak frequency	.0001	NS
	Peak frequency range	.0001	NS
	Average Power	NS	NS
	Energy	NS	NS
	Peak Amplitude	NS	NS
Component Under Peep	Duration	.0001	.041
	Max. frequency	.0001	.041
	Min. frequency	.0001	.044
	Max. - Min. frequency	.0001	.041
	Peak frequency	.0001	NS
	Peak frequency range	.0001	NS
	Average Power	.0001	NS
	Energy	.0001	.04
	Peak Amplitude	.0001	NS

DISCUSSION

The stated purpose of this study was to investigate changes in the structure of the peep call as female domestic chicks aged. Our results confirm Brückner's (1933) claim that structural changes occur with the vocalizations of the chick over time. Furthermore, we have shown at what ages these variations in structure occur. The chick produces the long peep during week one post-hatching and the two component peep during weeks two to four post-hatching. The three component peep is emitted during weeks four to six post-hatching and the component under peep is emitted from weeks six to ten. After ten weeks the female chick ceases to emit the peep call (Jennings, 1996).

In addition to a verbal description and spectrographic presentation of the different forms of peep call, a parameter analysis was conducted. The calls presented above (Figure 1) all displayed descending frequencies yet have different structural qualities. Collias (1987) stated that there are certain basic acoustic parameters or elements in fowl vocalizations that can be combined to produce vocal signals. The descending limb of the peep is the basic element of this call. Acoustic variation can be accounted for by additional components and their interaction with the descending limb. These changes might be accounted for by changes in syringeal anatomy as the chick ages. We investigated four different types of peep call and compared them over nine different acoustic parameters.

Results show that as the chick ages the calls display lower frequencies (max. frequency, min. frequency and peak frequency; Figure 1 and Table 1). In addition, peak frequency also decreases with age. Coupled with a decrease in the frequencies employed in the peep calls, parameter analysis also showed an increase in energy and peak amplitude (Tables 1 and 3). This would enhance call propagation (Chappuis, 1971; Morton, 1975) by concentrating the amplitude of the signal in lower frequencies. Analysis of variance showed a significant difference between the four call types over seven of the parameters analysed (duration, minimum frequency, peak frequency, peak frequency range, average power, energy, peak amplitude). The parameters of maximum frequency and difference between maximum and minimum frequency show that the call types occupy similar frequency ranges.

Jennings and Kent (1996) presented three different types of peep call that were found to be highly stable forms of vocalizing. Figure 2 shows that individual calls scatter closely around the group centroid for

the long peep. From the second week post-hatching chicks scatter further from the group centroids for the respective call types (two component peep, three component peep and component under peep). Correlations of the calls presented in this study show that as the chicks aged the calls became less stable. Therefore, a decrease in call stability is associated with an increase in the age of the chicks. Inter- and intra-individual variation in the calls of adult fowl are widely recognised (Konishi, 1963; Collias, 1987, Kent, 1987, 1989). This study demonstrates that inter-individual variation is high over different types of peep call and that intra-individual variation is relatively low with the exception of the component under peep (table 6). Despite the increase in individual variation, different types of peep calls could be accurately assigned to the correct type of call based on the discriminant analysis of the parameters examined in this study (Figure 2).

By maintaining a wide frequency range and sharp intensity changes as a component of the calls, the hen and brood mates may be capable of making binaural comparisons of the frequencies (Gulick, 1971; Konishi, 1973). This could facilitate location of the chick by the hen or conspecifics as the broody period declines and independent behaviour becomes established (Workman and Andrew, 1989). As the female chicks aged the structure of the peep call became more complex with increased complexity of call structures. In addition, the acoustic parameters of the peep calls changed and calls could be statistically discriminated upon on the basis of these changes. There was an increase in energy and peak amplitude, a decrease in minimum and peak frequency and the maintenance of wide frequency ranges.

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RAT BEHAVIOR IN THE EXPLORATION BOX - CLUSTER ANALYSIS

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ABSTRACT: The main objective of this study was to provide a description of the possible patterns of rat behaviour in the exploration box. Both age and sex of the animals were controlled. The measures taken assessed changes in the patterns of behavioural activity over time. Sex did not differentiate the rat's behaviour significantly. Two patterns of developmental changes were found. The results of cluster analysis with a sequential data sets lead to the conclusion that there are at least two levels of regulation of rat behaviour: activation and content organization. It was also shown that rat behaviour in the exploration box is not stereotyped but highly plastic. The results are discussed in terms of development, sex differences and individual differences.

The study of exploratory behaviour in the rat has implications for both the study of individual differences (Henderson, 1994; Pisula, Ostaszewski, Osiński, Trojan & Matysiak, 1995) and the development of psychological theory in general (Matysiak, 1992; Renner, 1990). There are several unsolved methodological issues in the study of exploratory behaviour. One is the selection of appropriate data analysis techniques. Another is the use of ecologically meaningful testing apparatus. The utility of multivariate analysis has been demonstrated in open-field behaviour (Pisula, 1994) and investigatory responses (Renner & Seltzer, 1994). It seems to be most useful in identifying behavioural strategies, particularly sequential analysis of behavioural transitions (Pisula, 1994; Renner & Seltzer, 1994). Thus it seems to indicate that the use of *a priori* indices are of little use. The use of multivariate techniques involving both quantitative and qualitative measures is more fruitful.

A second methodological issue concerns the appropriateness of the open field test. The complexity of behaviour manifested by the animal

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is, to some extent, a reflection of the complexity of the environment in which it functions. An open field test provides the animal with simplified conditions, which do not allow for initiating various forms of activity (Renner, 1990). Therefore, one may suppose, that the open-field behaviour is impoverished and does not reflect the complexity of behavioural repertoire characteristic for a given animal (Pisula, 1994). Thus, studies on individual differences manifested in spontaneous behaviour should involve test situations allowing animals to produce a greater diversity of behavioural acts. Such an apparatus was used in this study.

A third aim is to further investigate the behavioural correlates of the complex motivational mechanisms hypothesized to underlie exploratory behaviour (Matysiak, 1992). These correlates should reflect the complexity of the processes analyzed (Pisula, 1994).

The current study will also investigate sex and age differences with respect to exploratory behaviour. Both sex (Fitch & Dennenberg, 1995) and age differences (Bronstein, 1972; Oakley & Plotkin, 1975) have been often quoted. Renner, Bennet and White (1992) reported significant differences between rats of different age in various forms of exploration. However, their methodology was primarily aimed at investigatory and manipulatory responses. Therefore, it is important to confirm their findings using more general descriptors of exploratory behaviour.

METHOD

Subjects

Fifty five outbred Wistar rats were tested. The rats were grouped by sex with the following age breakdown; 5 subjects at 5 weeks old, 5 subjects at 10 weeks old, 5 subjects at 15 weeks old, 5 subjects at 20 weeks old. The male group had an additional 15 subjects at 25 weeks.

Apparatus

The box for exploratory behaviour measurement (Figure 1) was 42 x 32 x 40 cm. It was equipped with a see-saw, two ladders and two table tennis balls. All the things (except balls) were made of metal. Intensity of the white light (measured on the floor of the cage) was 50 lx.

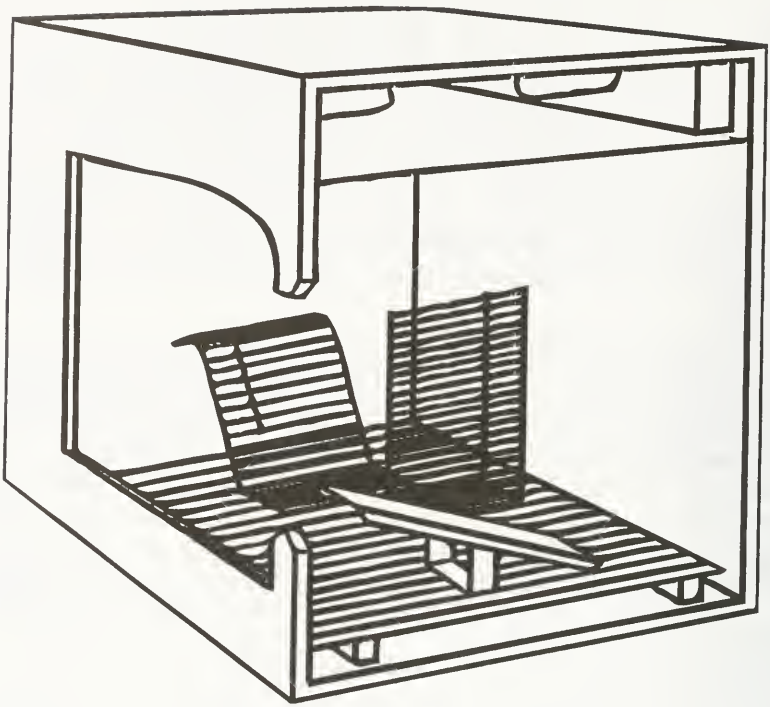

Figure 1. Exploration box used in this study.

Procedure

Each animal was placed individually in the exploration box for 60 minutes. All measurements were run between 6 to 8 pm under the light/darkness cycle 12/12h with turn on/off points at 8 am/pm. There was silence provided in the experimental room. Temperature was 20 ^{0}C. During the entire session the animal's behaviour was video recorded. The experimenter would leave the experimental room immediately after placing an animal in the exploration box.

Analysis of the video recordings

First, all behavioural acts were recorded in a two-dimensional table. The table reflected preceding (rows) and subsequent (columns) behaviours. Therefore, cell contents reflect the frequency of occurrence of a given sequence of behavioural acts. A trained observer analyzed the tapes frame-by-frame, classified behaviours and marked all of them

in the appropriate cells.

There were three tables constructed for each animal reflecting three periods of measurement: the first-5 minutes duration, the second following-15 minutes duration, and the last-40 minutes in duration. The periods of measurement were selected on the basis of exploratory data. The onset times of twenty two behavioural forms were included in the tables as follows: walking (w); running (r); jumping (j); rearing (re); leaning against the wall (l) - rat stands on its hind-quarters and makes one- or two paw contact with the wall; leaning against the object (lo) - rat stands on its hind-quarters and makes one- or two paw contact with the object; grooming (g); floor- (fs) or object- (os) sniffing - rat puts nose close to or in contact with the floor or object; air sniffing (as) - subject moves nose upwards at a considerable distance from any object and is standing with at least three paws on the floor; gnawing (gn); body scratching (bs); lying down (ld); freezing (f); lying rolled up (lr); object touching (ot) - rat makes contact with objects with single or two paws; object manipulation (om) - rat makes contact with objects with single or two paws causing its movement; climbing the ladder (c); sitting (s); stretching (st); crawling (sc). Tables constructed for 22 behavioural forms had 484 cells.

For the purpose of further analysis (cluster analysis), each cell was treated as a new variable. Therefore, the behaviour of each animal was described by three sets (reflecting three periods of the measurement), each consisting of 484 behavioural indices.

RESULTS

Sex and age differences

Comparisons between sexes were limited to the age categories where sex ratios were the same. An initial analysis showed that subgroup variance was not homogeneous. Distributions of most indexes were far from being normal. Therefore nonparametric procedures such as: the U Mann-Whitney, Kruskal-Wallis ANOVA and CHI-square were applied. The exception was grooming, for which an exact Fisher test was performed. Comparisons were conducted for all periods of measurement independently.

During the initial 5 minutes of the session the females took sitting position more often than the males [$U = 127$, $N = 40$, $p < 0.05$]. Analysis of variance conducted for the time spent on grooming, in this

period of testing, showed that the males groomed longer than the females [$F(1,30) = 6.0875$, $p < 0.05$]. During the next 15 minute period the females manifested gnawing more often than males [$U=114.5$, $N=40$, $p < 0.05$]. No sex differences were found during the last 40 minutes of the measurement.

Table 1 presents the age differences. Since the Kruskal-Wallis ANOVA was applied for this analysis, table shows ranked data for a given behaviour across time frames.

Table 1. Results of the analyses of age differences. Table includes only those behaviours that showed significant differences across time frames. The frequency of each behavioral act was ranked in ascending order. Cells show mean ranks in age subgroups. Expected ranking in each cell=20.5. Part A of the table shows the results of the first (5 min long) part of the session. Part B represents the next 15 minutes of the session and part C the last 40 minutes of the session. See "Procedure" for behaviour description.

Time	Behaviour	Mean Rank of Behaviour Frequency				χ^2	P
		5-weeks	10-weeks	15-weeks	20-weeks		
A	W	34.80	24.6	14.05	8.50	29.92	0.01
	L	23.45	26.45	19.40	12.7	7.84	0.05
	G	33.20	18.00	15.40	15.40	17.83	0.01
	FS	17.10	27.70	23.50	13.70	8.72	0.05
B	W	30.70	21.40	13.19	8.50	23.19	0.01
	LO	22.40	23.70	25.90	10.00	11.25	0.01
	FS	17.85	26.55	26.65	10.95	12.65	0.01
	AS	15.25	21.80	28.45	16.50	7.96	0.05
	GN	25.55	22.33	20.55	11.80	8.15	0.05
C	W	20.10	25.00	24.05	9.83	10.67	0.05
	LO	11.65	26.35	25.35	18.65	10.33	0.05
	FS	8.00	28.30	27.85	17.85	20.37	0.01
	OS	12.05	25.00	24.11	19.25	8.05	0.05
	AS	8.85	29.15	26.70	17.30	19.02	0.01
	GN	18.40	28.75	20.95	13.90	8.62	0.05
	S	12.00	18.35	28.55	23.10	10.91	0.05

Cluster analysis

Hierarchical cluster analysis (Ward's method) was conducted to identify the main behavioural patterns shown by the rats. The analysis

was run on the sequential data (484 cells of the transition table). There were three analyses conducted for three periods of measurements (1 per period). The results are summarized in Figure 2.

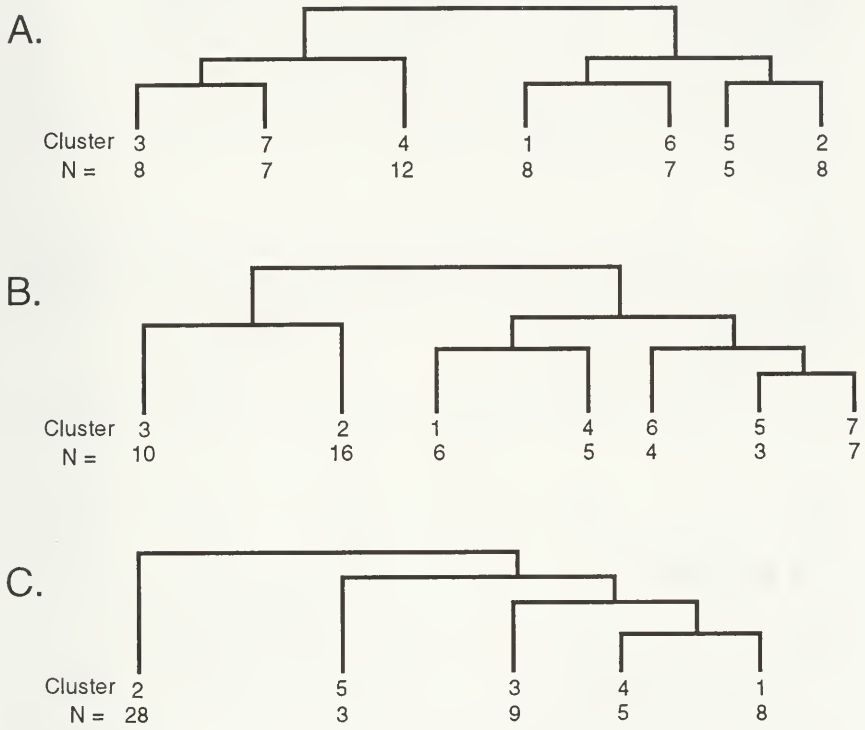

Figure 2. Inversed dendrograms illustrating results of cluster analysis of the experimental session, broken into three time periods: A., The initial 5 mins (0-5mins); B., the following 15 mins (5-20mins); and C., the last 40 mins (20-60 mins). At the ends of the branches, reflecting clustered groups of rats, are shown the cluster numbers and the cluster sizes (N).

The relationship between sex and cluster membership was analyzed using CHI-square statistics, assuming the ratio 20 females / 35 males. Table 2 shows the results of this analysis.

Age and cluster membership relationship were analyzed with Kruskal-Wallis ANOVA. The results are shown in Table 3. Figure 3 shows behavioural patterns presented by the extracted.

Each animal could belong to multiple clusters, because a separate cluster analysis was conducted for each time period. The cluster membership of each subject is provided below. Animals are described

with three digit codes. The first digit stands for cluster number extracted during the initial 5-min measurement, the second digit stands for following 15-min of the session, and the third one for third interval cluster. An "x" was used to indicate clusters with only one subject. These clusters are not shown in Figure 2. Cluster membership obtained is as follows: 11x, 111, 112, 123, 123, 124, 132, 141, 222, 222, 223, 242, 264, 272, 272, 272, 3x2, 311, 322, 322, 322, 322, 323, 332, 4x5, 422, 422, 423, 423, 425, 432, 432, 432, 432, 433, 454, 511, 54x, 562, 564, 564, 6x1, 6x3, 611, 643, 645, 651, 651, 732, 732, 732, 772, 772, 772, 772.

Table 2. Sex ratio in each cluster. Obtained Ratio, female/male proportion obtained in the clusters; Expected Ratio, expected ratio for a given cluster size.; * $p < 0.05$.

Cluster	Obtained Ratio			Expected Ratio		
	5-min	15-min	40-min	5-min	15-min	40-min
I	3 / 5	3 / 3	2 / 6	2.9 / 5.1	2.2 / 3.8	2.9 / 5.1
II	3 / 7	5 / 11	8 / 20	3.6 / 6.4	5.8 / 10.2	10.1 / 17.9
III	1 / 7	0 / 10	2 / 7	2.9 / 5.1	3.6 / 6.4	3.2 / 5.8
IV	4 / 8	2 / 3	5 / 0	4.3 / 7.7	1.8 / 3.2	1.8 / 3.2
V	5 / 0	2 / 1	1 / 2	1.8 / 3.2	1.1 / 1.9	1.1 / 1.9
VI	1 / 6	4 / 0	-	2.5 / 4.5	1.4 / 2.6	
VII	3 / 4	3 / 4	-	2.5 / 4.5	2.5 / 4.5	
χ^2 for difference between Expected and Obtained Ratios				12.37	14.55*	10.92*

Table 3. Mean age rank in extracted clusters. Age was ranked in a ascending order. Expected mean rank in each cell is 28. *p < 0.01.

Cluster	5-min	15-min	40-min
I	34.8	22.1	20.2
II	15.5	34.0	28.7
III	42.1	39.9	35.0
IV	34.9	25.2	17.2
V	15.5	15.0	21.8
VI	19.8	10.3	-
VII	23.7	5.5	-
χ^2	21.0*	34.9*	6.9

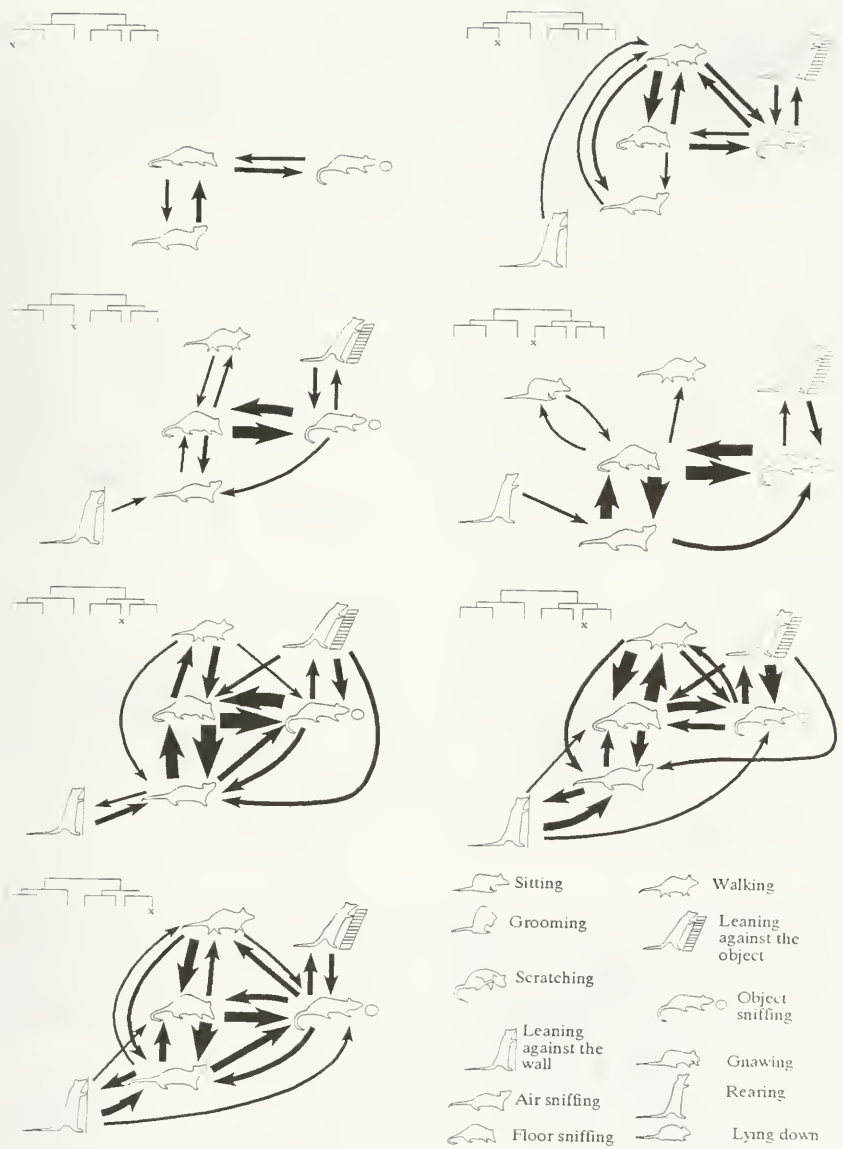

Figure 3a. Behavioral patterns during the first 5 minutes of the experimental session presented by rats of different clusters. The thickness of the arrows illustrates the frequency of the transition. Small dendrograms provided near the images inform about the cluster position within the whole sample.

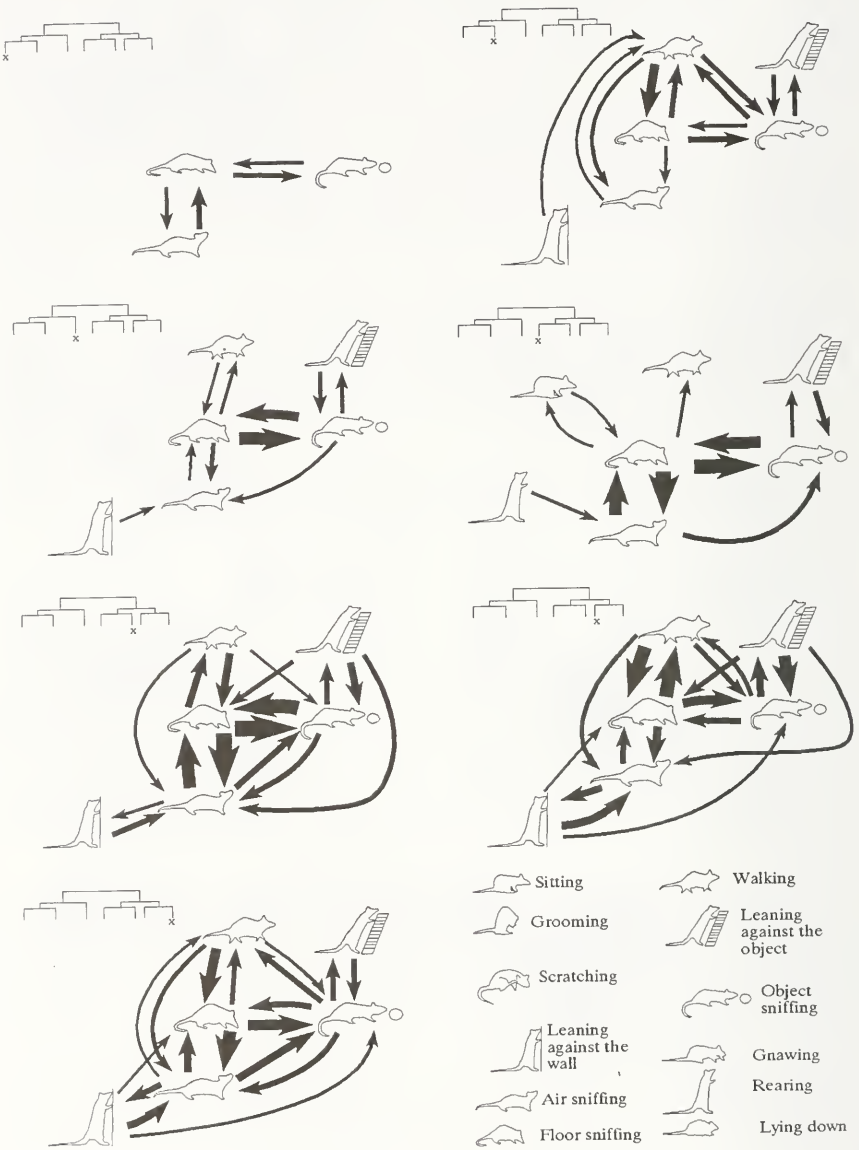

Figure 3b. Behavioral patterns during the 5-20 min period of the experimental session presented by rats of different clusters. The thickness of the arrows illustrates the frequency of the transition. Small dendrograms provided near the images inform about the cluster position within the whole sample.

Figure 3c. Behavioral patterns during the last 40 minutes of the experimental session presented by rats of different clusters. The thickness of the arrows illustrates the frequency of the transition. Small dendrograms provided near the images inform about the cluster position within the whole sample.

DISCUSSION

Sex and age differences

Gray (1971) found that female rats were more active and less fearful than the males. These results have been cited widely though they have lacked clear empirical confirmation. In a previous study conducted in our laboratory (Ostaszewski & Pisula, 1994) we analyzed

the individual differences in open field behaviour. In three strains (DA/Han, August, Long-Evans) females were found to be more active in the open field than males. In the case of WAG rats this relationship was reversed. Gray and Lallje (1974) also found similar effects. An interesting sex difference was found in grooming behaviour. Grooming was classified as "displacement activity" and is interpreted as an expression of internal conflict (Bindra & Spinner, 1958; McFarland, 1993). Thus, a longer time spent on grooming in males may reflect stronger internal conflict. It is still necessary to determine what motivational components, or what kind of mechanisms underlie this effect. It is probably a multilevel phenomenon. On the physiological level, female gonadal hormones may play a role (Denti & Negroni, 1975; Stewart & Cygan, 1980). On the other hand, male rats spend more time on competition and hierarchical behaviour than females (Colhoun, 1963). Experience of pain and losses may increase the general level of anxiety. Although some sex differences were found in this study, they do not appear to be as meaningful as developmental factors. This effect confirms the results obtained by Renner, Bennett and White (1992).

There were two main patterns of developmental change found in this study. The first one is a continuous decline of a given form of activity as the rats get older. This is most clearly seen in: walking, grooming and also gnawing. Conversely, activities such as: sniffing, leaning against the wall, leaning against the object, present quite different dynamics. In these cases, we can see an increase and subsequently decrease of the level of activity.

The developmental effects found in walking and grooming correspond with earlier findings. Bronstein (1972) found that open field activity in rats declined, beginning from the fifth week of life. Bronstein considered a square entered, when the head and at least one forepaw moved across the line. This, perhaps, is an insufficient measure. It leads to record ambivalent behaviour (moving head and forepaws forward and backward) as a locomotion. As a result of this procedure behavioural activities controlled by different motivational mechanisms may be combined. Nevertheless, a decrease of motor activity in rats after the fifth week of life is well confirmed. A developing rat presents the peak activity at the age of 20-30 days. This period is connected with maturation of the mesencephalon (Oakley & Plotkin, 1975) and intensive play (Colhoun, 1963).

A quite different effect was found with various forms of sniffing. The term "sniffing" is a simplification in this study. We included in

this category both vibrissae and nose working. Sniffing is directly involved in information seeking. The highest level of that activity was shown by 10- and 15-week-old rats. This result corresponds well with Renner's et al. (1992) work, which reported increase of sniffing behaviour in rats up to the age of 90 days. Hence, the subsequent decrease in this activity (after 15th week of life), found in this study is worth noting. A similar pattern was shown in leaning against the wall. Again, Renner at al. (1992) reported an increase in rats of age of 60 days, and subsequent decrease in older rats, which is in accordance with the results of this study, in which 10-week-old rats showed a peak in this activity. This pattern of developmental dynamics may reflect a great change in the rats' lives at this age. At the age of 70 days rats initiate sexual activity. At the age of 90-100 they are fully matured (Sokolov & Karasjova, 1990). This is a very stressful period in a rat's life. Males begin their struggle for a position in the colony. This process results in increased dispersal (Colhoun, 1963). Therefore, survival depends on the ability to recognize the threats coming from the environment. The observed decrease of sniffing in 20-week-old rats may be interpreted as a function of stabilization in the animal's development and adjustment to the environmental demands. This interpretation has to be verified experimentally.

The measures of contacts with objects indicated differential developmental trajectories. For example gnawing was found to decrease with age. Conversely, leaning, sniffing and leaning against the wall increased with age. Current data does not lead itself to an interpretation of those patterns. Further study is required in this regard.

Sex and cluster membership

The role of sex differences in cluster formation was not clear. There was no relationship between the sex and cluster membership during the initial five minutes of the session. The sex factor became apparent in the subsequent 15- and 40-minute time periods. It may be that in novel (often threatening) situations the mechanisms governing behaviour, common to all individuals, are decisively predominant. The role of the factors linked with sex increases as the organism adjusts to the environment.

Age and cluster membership

A strong relationship between cluster formation and the age of the

animals was found in this study. This effect corresponds well with the previous findings (significant age differences described above). Interestingly, the relationship between age and cluster membership disappeared in the 40-minute time period (see Table 3). One may suppose that developmental factors influence individual differences in behaviour in novel situations. Age dependent differences remained also in the third (40 min long) phase of measurement, but the relationship between age and cluster formations declined. This means that in a further phase of the measurement, besides the remaining behavioural quantitative differences among the animals of different age categories, the role of developmental factors in defining behavioural qualitative differences, which are also responsible for cluster formation, disappears.

Characteristic of cluster formation

Cluster formation indicated the presence of two main rat behaviour strategies. This was apparent in all the three analyses. Comparison of the left and right main (top) branches of the reversed dendrograms leads to the conclusion that the left ones reflect a lower level of overall activity, whereas the right ones illustrate a higher level of activity. Therefore, overall activity seems to be the main factor differentiating the animal's behaviour. This result corresponds with a previous, open-field finding (Pisula, 1994). However, the behaviour shown by animals in the exploration box in this study was far more complex than that in the open field. This effect supports our opinion that an open field test, in its widely used version, can impoverish the animal's behaviour. Thus, these data seem to support the idea that environmental complexity, understood as the potential complexity of relationships (contingencies) between the behavioural acts and changes in the environment, seems to be an important factor that influences behaviour (Kuo, 1967).

There seems to be no immediate relationship between the level of overall activity and qualitative pattern of behaviour in this study. Both high- and low- active clusters of rats presented complex behavioural patterns as well as extremely simple ones. These patterns differed in their content. On the other hand, there were patterns of similar content shown by the animals at a quite different level of overall activity. The regularities of the clustering process found can be summarized as the following three observations. Cluster formation involved both quantitative and qualitative differences in the animals' behaviour. The

primary factor in cluster formation is overall level of motor activity. At a relatively equal level of motor activity, the secondary (qualitative) differences influence the process of cluster formation.

The characteristics of cluster formation listed above allow me to formulate a view on behavioural processes governing rat behaviour in the exploration box. There are at least two levels of behaviour control processes. The first one is associated with arousal. There is general consent as to the neurophysiological basis of arousal: it is the function of the Reticular Activation System (RAS) of mesencephalon (Eysenck, 1967; Routtenberg, 1968). The classic work by Glickman, Sroges and Hunt (1964) supports this view. These authors tested the effects of lesions of different levels of central nervous system. The most evident changes in exploratory behaviour were obtained after mesencephalon lesions. On the other hand, Oakley and Plotkin (1975) showed that the highest level of activity in rats is strongly correlated with mesencephalon maturation.

The second level of behaviour regulation involves content organization. At this level, the direction of behaviour is determined. While in the first case the main point is to define an activational aspect of behaviour shown in every form of activity, in the case of the content analysis, it is important to orientate the behaviour with regard to the external environmental stimulus, to select the form of behaviour, and to allocate it in time. This level of behaviour regulation seems to be based on the limbic system (Routtenberg, 1968) and frontal cortex (Kolb, 1984). Therefore it is a higher level of regulation, linked with phylogenetically younger parts of CNS.

Renner and Seltzer (1994) discussed their finding in terms of a possible stereotyped nature of exploratory behaviour. They presented the data supporting the view that, at least in rats, exploratory behaviour is definitely not stereotyped. The results of cluster analysis obtained in this study also support this view. Though rats formed certain types of behavioural activity, reflected by extracted clusters, it is hard to say that they were stereotyped. The analysis of the flow of rats between clusters extracted in different periods of measurement leads to the conclusion that they can utilize different patterns of behaviour at different times and at different levels of general activity. Thus, the presence of an inherited mechanism governing the structure of the behaviour in the exploration box seems to be unlikely.

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EFFECTS OF AGING ON INHIBITORY LEARNING AND SHORT-TERM MEMORY IN *DROSOPHILA MELANOGASTER*

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ABSTRACT: Two experiments have been performed in young (7 days old), middle-aged (28 days old) and old (49 days old) *Drosophila melanogaster*. In Experiment 1, the inhibitory conditioning of the Proboscis Extension Response (PER) to sucrose was displayed under three Inter-Trial-Interval (ITI) schedules: 1, 2 or 4 minutes. The results did not reveal any age-related impairment of short-term-memory. The PER suppression performance was higher in middle-aged and old flies than in young ones, whatever the ITI. In Experiment 2, the habituation of the PER to sucrose was induced to investigate the hypothesis of an age-related increase of the non associative processes involvement (sensory adaptation, motor fatigue) in the PER suppression. The results showed that once such peripheral effects were removed, suppression performances no longer varied with age.

RESUME: Deux expériences ont été réalisées chez des drosophiles jeunes (7 jours), d'âge moyen (28 jours) et âgées (49 jours). Dans l'expérience 1, l'inhibition conditionnée de la Réponse d'Extrusion du Proboscis (REP) au sucre a été étudiée sous trois conditions expérimentales différant par la durée de l'Intervalle Inter-Essais (ITI): 1, 2 ou 4 minutes. Les résultats ne révèlent pas d'atteinte liée à l'age de la mémoire à court terme. Les performances de suppression de la REP sont plus élevées chez les mouches d'âge intermediaire et âgées que chez les jeunes, quel que soit l'ITI. Dans l'expérience 2, l'habituation de la REP au sucre a été étudiée pour tester l'hypothèse d'une augmentation liée à l'âge de l'implication de processus non-associatifs (adaptation sensorielle, fatigue motrice) dans la suppression de la REP. Les résultats montrent que lorsque de tels effets périphériques ne sont pas en jeu, les performances de suppression ne varient plus avec l'âge.

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Over the last few decades, *Drosophila* has been one of the principal invertebrate model in the experimental gerontology area. Although mammals provide biological models closer to human beings, their use encounters some sensitive variables (motivation level, psychomotor abilities) which make the study of age-linked variations of biological or behavioral traits more difficult. The *Drosophila* fruitfly offers several major advantages as an experimental model since the generation time is short, the reproductive capacity is high and its genetic, physiological and biochemical characteristics are well-known. Especially in experimental gerontology research, the main practical benefit of the use of *Drosophila* lies in its short life duration which remains below three months with the standardized rearing-temperature of 25°C.

Indeed, many studies dealing with the age-linked changes at different levels of organization (molecular, cellular, physiological) have already been performed on *Drosophila*. We already know that the biological characters related to fitness (viability, reproductive activity) decline with age (Review in Boulétreau-Merle, 1988), as well as some behavioral capabilities, such as the spontaneous locomotor activity rate or the ability to climb up a vertical surface (Review in Le Bourg et al., 1993). Some studies have also been devoted to the search of possible age-linked changes in learning abilities. A single experimental situation has been used until now, based upon the conditioning suppression of the Proboscis Extension Response (PER) to sucrose. The authors (Brigui et al., 1990; Fresquet and Médioni, 1993) showed that the ability to suppress the PER, and so to reach a learning criterion, decreased after a middle-age (28 days old). Moreover, in a more simple learning situation, such as the habituation of the PER to sucrose, where no associative component is involved, the acquisition is delayed as well after middle age (Fois et al., 1991). Taken together, these results suggest that aging specifically impairs the central inhibitory abilities, making middle-aged and old flies less able to suppress an unconditioned response, whereas associative capacities might be preserved from major aging damages.

The age-linked deterioration of inhibitory capacity has also been reported in rodents, where aged animals have greater difficulty suppressing a response in passive avoidance learning situations (Lamberty and Gower, 1990; Fagioli et al., 1992). Even in humans, the "behavioral rigidity" often associated with aging, consisting in an increasing inability to modify previously learned or habitual behavioral patterns, could be ascribable to a lack of inhibition (Dean and Bartus,

1988).

Therefore, age-related damages in central inhibitory mechanisms seem to be wide-spread across species. Likewise are age-related impairments in Short-Term-Memory (STM) often observed. By using a multi-trial maze learning tasks where the Inter-Trial-Interval (ITI) duration can be lengthened, some studies in rats (Soffié and Giurgea, 1988; Dellu et al., 1992) showed that the longer was the ITI, the slower did old animals learn.

The purpose of this *Drosophila* study is to find out whether any age-related change may occur in Short-Term-Memory. A decline could be inferred if the acquisition levels reached by old flies decrease as the ITI duration increases, whereas the performance of younger flies remains little or none variable. By using the same inhibitory conditioning as previously done (Brigui et al., 1990; Fresquet and Médioni, 1993), we are able to vary the ITI and so, to compare the performance reached by several age-groups at different ITI schedules. Our learning procedure is an anterograd pavlovian conditioning (Médioni and Vaysse, 1975) based upon the systematic association between the Unconditioned Stimulus (US - Sucrose) and a negative reinforcer (Quinine). The US induces the PER while the quinine acts right after as the punishment of the response releasing. The conditioned inhibition of the PER to sucrose improves over trials as the learning of the association between the US and the negative reinforcer develops.

The previous studies (Brigui et al., 1990; Fresquet and Médioni, 1993) showed that middle-aged (28 days old) and old flies (49 days old) have greater difficulty learning the conditioned inhibition to sucrose, compared to young flies (7 days old). Either the acquisition speed, measured as the number of trials needed to reach an acquisition criterion, or the final acquisition level, measured as the total number of PER suppressions, decreases after middle-age and no longer changes to the age of 49 days old. In the present experiment, the learning of the conditioned inhibition will be tested at various ITI durations and an aging effect on STM may be revealed if the differences in the level of acquisition attained by young (7±2 days old), middle-aged (28±2 days old) and old flies (49±2 days old) increase as the ITI lengthens.

In a second experiment, the non-associative learning of the habituation of the PER to sucrose will be used to assess the possible role of various peripheral mechanisms (sensory adaptation, motor fatigue...) in the PER suppression. Experiment 2 will be designed to determine whether non-associative processes become more important with age.

EXPERIMENT 1 - METHODS

Subjects and apparatus

Adult *Drosophila melanogaster* males of the wild-strain "Meyzieu" (France) were maintained by mass-mating on a standard nutritive medium (agar, sugar, corn-meal and killed yeast) enriched with live yeast. Eggs from parents 4-5 days old, laid for approximately 15 hours, were set in batches of 25 into 80-ml vials supplied with 15 ml of the usual medium and live yeast. After 9-10 days of development, virgin males were transferred after etherization, in batches of 15, to vials containing the S.101 synthetic medium of Pearl et al. (1926) enriched with live yeast and renewed twice a week. Flies were reared in the experimental room under controlled temperature (25±0.5°C), relative humidity (85±5%) and photoperiod (L:D = 12:12; 250 lx).

Under such rearing conditions, no significant mortality (lower than 5%) was observed until 30 days old and plotting the longevity curve (see Figure 1) reflects a normal aging pattern in the sample (rectangular shaped curve), excluding accidental or premature deaths. In males, the mean longevity (50% of the sample still alive) is 49.26±1.01 days and the maximum longevity is 73 days (n = 150).

In each experiment, three age groups were compared: young (7±2 days old), middle-aged (28±2 days old) and old (49±2 days old). Any variation detected in the characters under study between young and middle-aged flies could be attributed to a true aging effect, since no significant differences in mortality rate were observed between these two ages. By contrast, any change observed between young or middle-aged flies and old ones might be related either to a true aging effect, or to a selective mortality linked to the behavior under study (see Figure 1).

The conditioning device had been described in detail by Médioni and Vaysse (1975). One fly was tested at a time, walking at a fixed point on a dark-shaded revolving drum (4 mm/s) continuously rinsed with distilled water. The black pathway was interrupted at six equidistant points where white rectangular areas of Whatman® filter paper soaked in stimulating solutions were laid down. When the fly walked across the filter-paper, the solutions were detected by its tarsal chemoreceptors, so releasing the PER. The solutions remained out of reach of the proboscis and could not be ingested. The fly being trained was observed through a stereomicroscope (×25) and any proboscis extension, whether complete or not, was scored as a response.

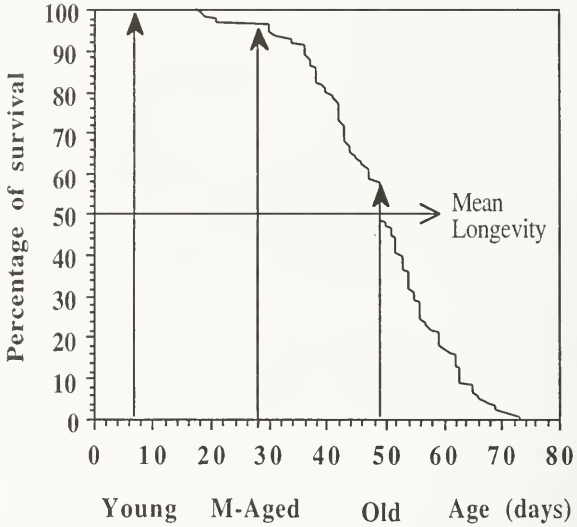

Figure 1. Longevity curve of the males of the wild-strain Meyzieu reared at 25°C. The correspondence between the rate of mortality and the age-group tested is shown.

Procedure

Under weak ether anesthesia, experimental flies were affixed by the notum to the tip of a needle with a droplet of synthetic varnish. The needles were then stored within a closed moistened box (100% Relative Humidity) where flies underwent an hydric diet for an average of forty five hours (see Results Section). This deprivation period was required for the PER to be unconditionally released by sucrose.

Thereafter, the response threshold to sucrose was individually determined with a procedure derived from the psychophysical method of increasing intensity of stimuli. Each fly was stimulated with an ascending scale of sucrose molar solutions (1/512 mol, 1/256 mol, 1/128 mol, 1/64 mol, 1/32 mol, 1/16 mol, 1/8 mol). The range of effective concentrations was first determined roughly by using a two-step increase between two consecutive stimulations. Two trials per concentration were given at a 1-min inter-trial-interval if either no PER or two PER were observed, but three trials if only one response was released. The threshold was considered as crossed when two responses occured. Then the just lower concentration, which was omitted in the two-step increase, was tested and considered as the threshold value if 2 PER were still observed. The flies which responded to the minimal dilution 1/512 mol were rejected because their threshold remained

unknown (≤ 1/512 mol) and their physiological state was usually poor. Otherwise, a sucrose solution eightfold more concentrated than that of the individual threshold was used during the training session. Hence, flies which did not respond to the 1/8 mol dilution were discarded because the 1 mol value was the highest usable sucrose solution. With such a procedure, using a threshold constant multiple, all the experimental subjects could be trained with a PER releaser of approximately the same effectiveness, if not of equal physical intensity.

Pre-training: One minute after the response threshold determination, each fly was submitted to four pre-training trials (1 min ITI), each including a sucrose stimulation immediately followed (within 2.5 s) by a distilled water neutral stimulation (Médioni et al., 1978). Flies which failed to respond to each pre-training trial, as well as subsequently to the first training-trial, were discarded. This preliminary stage allowed us to reject animals precociously affected by non-associative processes (sensory adaptation, motor fatigue, habituation to sucrose) leading to the disappearance of the PER to sucrose.

Training: One minute after completing the pre-training, the learning session started for 24 acquisition trials arbitrarily defined as 6 consecutive 4-trial blocks. For each trial, the fly was walking at a fixed-point on the humid pathway for approximately 30 seconds as the drum turned and then it was stopped for a rest interval until the next trial. At half-way, the fly crossed a paper soaked in a sucrose solution and right after (within 2.5 seconds) another one soaked in a 10^{-1} mol quinine chlorhydrate solution (Negative Reinforcer).

The conditioning was displayed according to three acquisition schedules differing by the ITI duration: 1, 2 or 4 minutes. Note however that the duration of the rest period between two consecutive trials varied accordingly but not the fixed-point walking time.

Sixteen young (7±2 days old), middle-aged (28±2 days old) and old flies (49±2 days old) were tested in each acquisition schedule (three ITI), giving nine experimental groups.

The individual acquisition performance of the conditioned inhibition was the number of PER suppressions recorded either at the end of the 24 training trials, or for each 4-trial block.

RESULTS

Duration of deprivation and threshold to sucrose

The deprivation times required to meet the threshold criteria varied with age: $F(2, 141) = 15.82$, $p < 0.0001$. Young flies had to be deprived longer than middle-aged and old ones: 49.69 hr (± 0.45 SE), 41.01 hr (± 1.34 SE) and 42.48 hr (± 1.44 SE) respectively. This result showed that the sensitivity to inanition increased noticeably with age, being most probably linked to the general physiological state.

The response threshold to sucrose also varied with age: $F(2, 141) = 10.12$, $p < 0.0001$. The mean threshold value was close to 1/32 mol in 7-day-old flies and increased to 1/16 mol in older flies. This age-related decrease in reactivity to sucrose might be due to the loss of some gustative tarsal chemoreceptors, as suggested by Stoffolano (1975) in the blowfly *Phormia regina*. Consequently, the proportion of flies discarded for a threshold to sucrose that was too high (> 1/8 mol) increased with age: χ^2 (2) = 33.80, $p < 0.0005$, whereas the number of flies discarded for a threshold that was too low (≤ 1/512 mol) decreased with age: χ^2 (2) = 11.66, $p < 0.001$ (see Table 1). Note however that these age-related differences in the reactivity to sucrose were eliminated during the training by stimulating all the flies with a constant multiple of their threshold.

The threshold values (mmol) and the fasting durations were not correlated at any age: young group, $r(48) = - 0.14$, ns; middle-aged group, $r(48) = + 0.13$, ns; old group, $r(48) = + 0.05$, ns.

The number of flies discarded for failing to respond at each pre-training trial was very low whatever the age (see Table 1).

Table 1. Number of discarded flies before the training in Experiment 1.

Criterion of elimination	Age (in days)		
	7	28	49
Threshold > 1/8 mol	38	68	167
Threshold ≤ 1/512 mol	23	8	5
Pre-training selection	2	1	1

Suppression performance

We have verified first at the individual level that the conditioning

scores were neither correlated with the fasting durations nor with the threshold values. A three-way analysis of variance with two main factors (Age and ITI) and one repeated factor (Trials) showed that both Age and Trials had significant effects on the number of PER suppressions: for the Age effect, $F(2, 135) = 10.69$, $p < 0.0001$, and for the Trials effect, $F(5, 675) = 27.04$, $p < 0.0001$. The interactions between Age and Trials on the one hand, ITI and Trials on the other hand, also had significant effects on the number of suppressions: for the Age × Trials interaction, $F(10, 675) = 6.31$, $p < 0.0001$, and for the Trials × ITI interaction, $F(10, 675) = 1.99$, $p < 0.04$.

This analysis revealed that the suppression performance increased with age, whatever the ITI duration. Even though a decrease might be noticed in the final acquisition level reached by 49 days old flies under the 4 minutes ITI schedule, such difference was not statistically significant (see Figure 2).

On the other hand, the suppression performance improved across trials in the three age-groups while oldest flies remained able to display the highest number of suppressions throughout the training (see Figure 3).

Finally, a deleterious effect of the lengthening of the ITI might be established by analysing the learning curves. Indeed, the acquisition was delayed, in the three age-groups, when the ITI reached 4 minutes (see Figure 4).

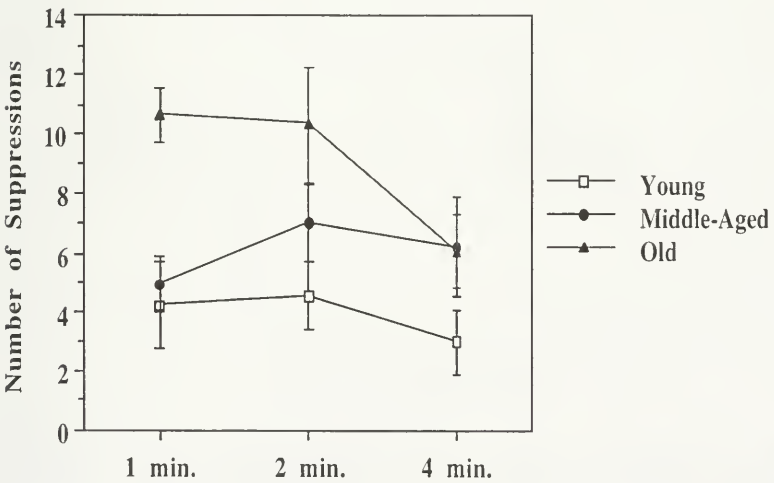

Figure 2. Experiment 1: Mean (and SE) acquisition performance as a function of age and Inter-Trial-Interval (abscissae), at the end of the training.

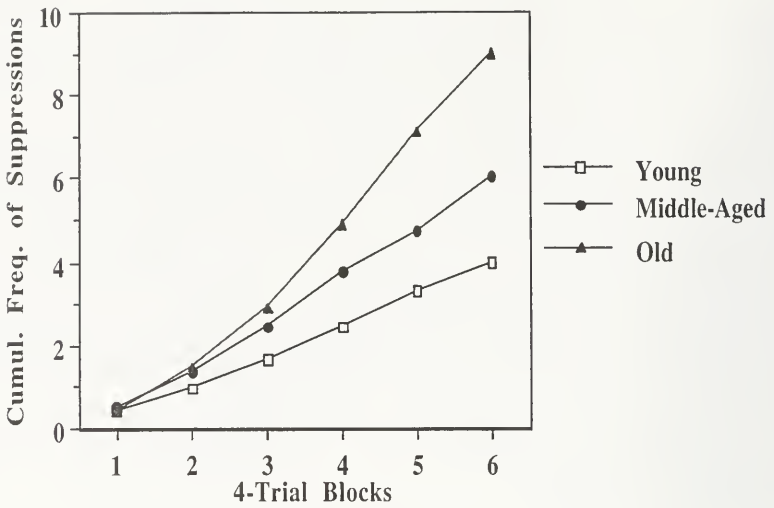

Figure 3. Experiment 1: Cumulative frequency of suppressions all along the 24 training trials, as a function of age.

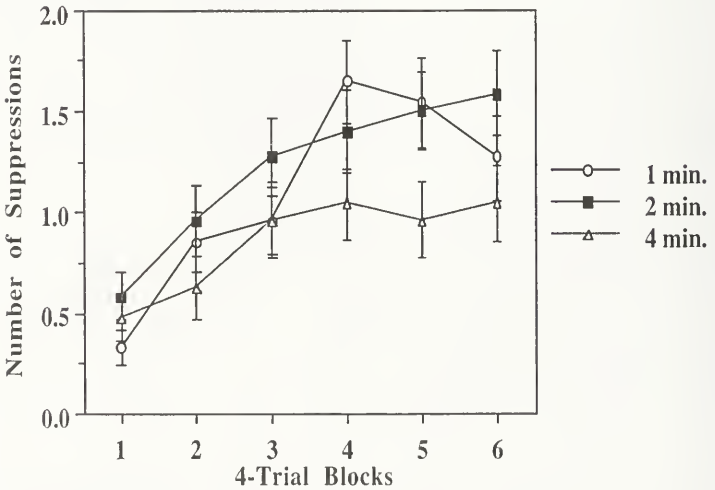

Figure 4. Experiment 1: Mean (and SE) acquisition performance all along the 24 training trials as a function of Inter-Trial-Interval, the three age-groups being pooled.

DISCUSSION

The results of this experiment are quite surprising, mainly due to the age-linked increase in the acquisition performance. Such an age-related variation has not before been reported, as for example in Brigui et al. (1990) who showed in middle-aged and old flies a greater difficulty in acquiring the inhibitory conditioning. Also, an age-related decrease in the ability to suppress the PER to sucrose was stated either in a visual discrimination inhibitory conditioning (Fresquet and Médioni, 1993) or in habituation learning (Fois et al., 1991). These previous experiments were all performed thru the same experimental procedure, including first a determination of the threshold to sucrose then a pre-training, prior to the learning session. Therefore, in all these studies a certain pourcentage of the initial samples had to be discarded on the basis either of the threshold value or of the pre-training score, as we did in this first experiment. Indeed, from the beginning of Experiment 1, the proportions of flies that were kept until the end of the learning session amounted respectively in the young, middle-aged and old group to: 43.24%, 38.40% and 21.72%. These proportions are very close to the ones reported in Fresquet and Médioni (1993) where the acquisition scores were computed on 47.06% of young flies, 26.66% of middle-aged flies and 23.88% of old flies. Such data were not published in the two other studies (Brigui et al., 1990; Fois et al., 1991) but we may assume that the outcomes are quite the same since the experimental design was not much different. Consequently, the prerequisite selection could not account for the difference between Experiment 1 and the other studies of reference in the variation observed with age.

From the previous experiments dealing with inhibitory conditioning, authors concluded that the central inhibition mechanisms were the target of aging damages, excluding any possible impairment of the associative processes. Indeed, the evaluation of the efficiency of associative and inhibitory processes could be dissociated in the discrimination procedure (Fresquet and Médioni, 1993) and only the latter were impaired with age. Moreover, in the habituation task (Fois et al., 1991) where no associative component was involved, the age-related decline in the suppression ability was observed again from a middle age.

The purpose of Experiment 1 was as well to investigate a possible age-related change in short-term memory. One might indeed expect, if any impairment in short-term memory occurs with age, that the age-

related differences in acquisition will increase with the lengthening of the ITI. No such result was obtained since neither the duration of the ITI itself, nor the ITI by Age interaction, had a significant effect on the acquisition levels. Even though the performance level reached by the oldest group decreased noticeably with the 4-min ITI, it remained higher, at least, than the mean performance level of youngest animals.

However, a damaging effect of the lengthening of the ITI was revealed by analysing the course of acquisition, which was delayed under the 4-min ITI. Since the speed of acquisition was decreased to the same extent in the three age-groups under this time-condition, no age-related differences in the efficiency of memory could be put forward. Otherwise, it is worth noting that the acquisition curve is the most regular and reaches its highest value with the 2-min ITI whatever the age. These observations may remind the studies made by Jost (1897) and Piéron (1909) on habituation in invertebrates, showing that optimum intervals allow more rapid acquisition and better retention, whereas shorter or longer ITI yield a decrease in these performances. We thus would assume that the 2-min ITI is more convenient for acquiring such inhibitory conditioning when compared to a shorter (1-min) or a longer (4-min) interval. Indeed, Médioni (1986) had previously reported higher suppression performances in young (7 days old) *Drosophila* tested with a 2-min ITI compared to 1 minute.

EXPERIMENT 2

Experiment 2 was undertaken to test whether peripheral, uncontrolled processes may have influenced the age-linked increase in the number of suppressions previously reported in Experiment 1. For that purpose, the three age-groups were tested in the habituation of the PER to sucrose as following a procedure which included single sucrose stimulations (not associated with a negative reinforcer).

Habituation may be defined as the waning of the PER to the monotonous repetition of the sucrose stimulation. However, once the PER is no longer released after a variable amount of training, one still needed to establish the nature of the mechanisms underlying the disappearance of the response. Indeed, we made the difference between "true habituation" which refered exclusively to a centrally controlled stoppage of the PER and "pseudo-habituation" which involved peripheric mechanisms such as sensory adaptation to sucrose or motor fatigue of the proboscidian effectors. Previous studies in *Drosophila*

have indeed demonstrated the central origin of habituation process. Duerr and Quinn (1982) induced habituation by applying sucrose stimulations to the right prothoracic tarsus and showed that responsiveness was depressed to subsequent stimulations to the left prothoracic tarsus for at least 10 minutes. These authors affirmed that the interaction between stimuli to contralateral legs took place centrally. Thereafter, Bouhouche et al. (1993) used an habituation procedure based on ispsi- and contralateral sucrose stimulations to compare wild-type and mutants *Drosophila*, these latter being characterized by a disorganized protocerebral bridge and a partial loss of the fibers connecting the central complex with the central brain. After having checked that motor activity and sucrose reactivity were normal in mutants, authors reported abnormally low habituation scores. These results were interpreted throught a defect in processing the information between the two brain hemispheres and such a reduced inter-hemispheric communication impaired the ability to inhibit the PER when stimulations were unilaterally applied.

No such habituation procedure involving unilateral stimulations was used in Experiment 2 but instead, a dishabituation test was performed at the end of the training. The presentation of a dishabituating stimulus (yeast smell) simultaneously with the sucrose stimulation disclosed the origin of the disappearance of the PER during training: its re-appearance in the dishabituation test revealed the involvement of central processes, mediating a "true habituation" learning; if the PER was not restored during the dishabituation test, then we could reliably infer that peripheral mechanisms were mainly involved in the PER disappearance and that animals were just no longer able to display a proboscis extension ("pseudo-habituation"). Such peripheral mechanisms could be sensory adaptation to sugar and/or a muscular fatigue of the proboscidian effectors.

The comparison, across the three age-groups, of the respective proportions of truly habituated vs. pseudo-habituated flies has permited ascertaining whether the role of peripheral mechanisms in the PER suppressions became more important with age. If such a positive relation could be established, then the higher numbers of suppressions reported with age in Experiment 1 might not reveal a higher efficiency of the inhibitory abilities.

METHOD

Subjects and apparatus

The experimental subjects came from the same wild-strain (Meyzieu) and were reared in the same conditions as in Experiment 1. Also, same apparatus was used.

Procedure

Threshold determination: After the deprivation period was completed, the individual threshold to sucrose was measured by using a simpler procedure derived from that of Experiment 1. It was still based upon the psychophysical method of increasing intensity of stimuli except each sucrose concentration was tested only once in order to minimize the number of pre-exposures to this stimulus. Subsequently, the sucrose concentration used during training was only twofold higher than the individual threshold, in agreement with one of the parametric characteristics of habituation learning defined by Thompson and Spencer (1966): "the weaker the stimulus, the more rapid and/or more pronounced is habituation. Strong stimuli may yield no significant habituation".

Therefore, the animals were discarded before training either if they responded to the minimal concentration tested (1/512 mol) because their physiological state was usually poor and their threshold remained unknown, or if they did not respond to the highest concentration (1/2 mol).

Training: One minute after the threshold measurement was completed, the habituation training began for, at most, 32 training trials (i.e. eight four-trial blocks) separated by a 1 minute intertrial-interval. Each trial included a sucrose stimulation followed, within 2.5 seconds, by a distilled water stimulation. The training was stopped as soon as a criterion of acquisition defined as 3 PER-suppressions within a 4-trial block was reached. Flies which failed to reach the acquisition criterion within 32 training trials were considered as non-learners. Otherwise, the dishabituation test was performed one minute after the end of the training. The first dishabituation trial included the sucrose stimulation and a simultaneous diverting event (yeast smell). If the PER was then restored, the fly was considered as "truly habituated" since we might reliably infer a central leading to the suppression of the response. If no PER was observed, a second trial was presented one minute later,

including the single sucrose stimulation, and the fly was considered as truly habituated if the PER was then elicited. If no PER had been released, then the peripheral mechanisms (sensory adaptation, motor fatigue) were believed to have caused the response disappearance during training and no habituation learning was assumed. These flies were designated as "Pseudo-habituated".

The experiment went on until 16 truly habituated young (7 ± 2 days old), middle-aged (28 ± 2 days old) and old flies (49 ± 2 days old) were collected. The experimenter was blind to the age of the fly during experiment.

RESULTS

Duration of deprivation and threshold to sucrose

Twenty-nine to thirty hours of deprivation were needed on the average, in each age-group, to meet the threshold criteria: $F(2, 107) = 2.22$, ns. The durations of deprivation recorded here were shorter than those in Experiment 1 (above forty hours). This difference might be explained by the higher threshold limit accepted in Experiment 2 (until 1/2 mol against 1/8 mol in Experiment 1).

The response threshold to sucrose did not vary with age: $F(2, 107) = 1.35$, ns, the mean threshold value being close to 1/8 mol in the three age-groups. We noticed however a higher proportion of young and old flies discarded for a too high reactivity threshold (> 1/2 mol), compared to middle-aged ones: $\chi^2 (2) = 11.12$, $p < 0.0001$ (see Table 2).

Table 2. Number of discarded flies before the training in Experiment 2.

Criterion of elimination	Age (in days)		
	7	28	49
Threshold > 1/2 mol	33	6	30
Threshold ≤ 1/512 mol	4	2	5

The absence of age-linked variation in the durations of deprivation required or in the thresholds to sucrose might be related to the use of the simpler threshold measurement (a single trial per concentration) since the more extensive procedures previously used, in Experiment 1 as well as in Brigui et al. (1990), allowed such variation to be reported.

The threshold values (mmol) and the durations of deprivation were

not correlated when the data of the three age-groups were pooled: $r(144) = +0.15$, ns.

Distribution of experimental subjects

Of the 110 flies which completed the experiment, 24 (21.82%) failed to reach the acquisition criterion (see Table 3). The proportions of these non-learners did not differ in age: $\chi^2 = 2.44$, ns. These flies, most probably, were slower to learn the habituation but might not have been affected by peripheral mechanisms since they continued to respond frequently during training.

On the other hand, 38 flies (34.54%) fell into the "pseudo-habituated" category and their proportions in each age-group were not significantly different: $\chi^2 = 3.73$, ns (see Table 3). Even if a weak increase might be noticed in the proportion of oldest pseudo-habituated flies, our result did not allow us to conclude that the involvement of peripheral mechanisms in the PER suppression increased with age.

Table 3. Distribution of the experimental subjects at the end of the training in Experiment 2.

Categories	Age (in days)		
	7	28	49
Habituation	16	16	16
Pseudo-Habituation	9	10	19
Criterion not reached	11	6	7

Habituation performance

A one-way analysis of variance performed on the three truly habituated samples did not reveal an age-linked variation in the number of trials needed to reach the acquisition criterion: $F(2, 45) = 0.46$, ns (see Figure 5).

The habituation performance (number of trials to criterion) was not correlated with the durations of deprivation nor with the threshold values (the data of the three age-groups being pooled).

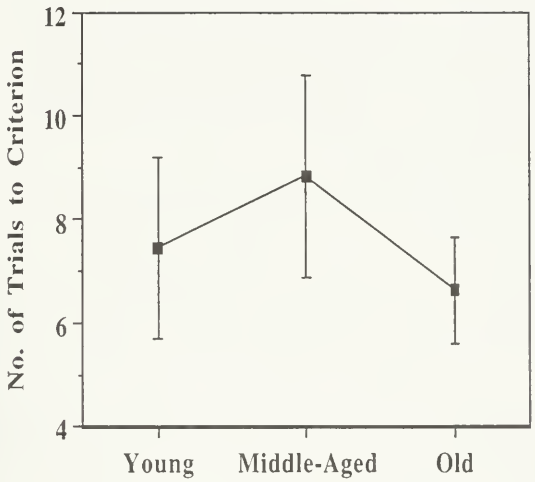

Figure 5. Experiment 2: Mean (and SE) number of trials needed to reach the acquisition criterion in the truly habituated groups, as a function of age.

DISCUSSION

Experiment 2 was undertaken to study the role of peripheral mechanisms (sensory adaptation, motor fatigue) in PER suppression. In consideration of the results of Experiment 1 revealing an age-related increase in the number of PER suppressions, we needed to determine the proportion of suppressions ascribable to central processes vs. that to peripheral ones in order to learn whether these latter might induce an

increasing proportion of suppressions as flies age. The results of Experiment 2 revealed that the number of pseudo-habituated flies did not vary significantly with age, leading us to conclude that peripheral mechanisms are not of greater importance in aged flies. A slight increase however was noticeable in the proportion of oldest pseudo-habituated flies but it does not induce a significant variation on the basis of the samples collected.

Up to now, the only studies dealing with that question in a similar learning situation are from Médioni and Vaysse (1975) and Vaysse and Médioni (1976). These authors established in young flies (7 days old) a ratio of approximately 1 suppression out of 5 ascribable to a sensory adaptation to sucrose, as well as 20% derived from pseudo-conditioning

effects, i.e. from the single repetition of the negative reinforcer unless it was associated with the sucrose stimulation. Ultimately, 40% of suppressions may be related to peripheral factors in young flies. Such a proportion is much too large in itself and would badly affect the results if it were to increase with age.

Finally, Experiment 2 also allowed us to compare the habituation performances across the three age-groups. Even if more simple than the inhibitory conditioning since no associative component is involved, habituation refers to central abilities as cleared from the effects of peripheral mechanisms. No age-linked variation could be stated in the ability to reach the acquisition criterion. This result might be put in relation with an hypothesis of a rising influence of peripheral mechanisms in aged flies because their suppression performances are no longer higher than those of younger flies as soon as the incidence of any peripheral factor is removed. On the other hand, this result is not in accordance with a previous one (Fois et al., 1991) showing a decrease in the habituation speed from a middle age.

GENERAL DISCUSSION

Two experiments have been performed in young, middle-aged and old *Drosophila melanogaster*. Experiment 1 aimed at investigating possible aging effects on short-term-memory abilities required in the inhibitory conditioning acquisition. No significant decrease in performance was produced in aged flies by lengthening the ITI, preventing us from claiming any impairment of short-term-memory. Only a slight decrease was noticeable in the oldest group when the ITI reached 4 minutes, which might let us expect that the use of longer intervals might reveal some memory deficiencies. Damaging effects of aging on memory processes have been reported in mammals either in maze learning (Soffié and Giurgea, 1988; Dellu et al., 1992; Lindner et al., 1992) or in discrimination learning (Goodrick, 1968; Bartus et al., 1978; Cavoy and Delacour, 1993). In these studies, the differences across age-groups in acquisition levels increased as the ITI was lengthened. In invertebrates, a study performed in the nematode *C. elegans* (Beck and Rankin, 1993), based upon a mechanosensory reflex habituation learning, led to opposite results since old animals displayed the higher acquisition rates with the longest ITI.

On the other hand, Experiment 1 revealed an age-linked increase in the number of PER suppressions, whatever the ITI duration. Such a

result was not consistent with the ones of previous studies in *Drosophila* dealing with comparable conditioning procedures (Brigui et al., 1990; Fresquet and Médioni, 1993) where higher suppression performances were reported in young flies. Literature in rodents as well gives evidence of an age-linked impairment of central inhibitory abilities. Indeed, some studies in mice or rats have shown the increased difficulties of old animals to learn a passive avoidance task (Lamberty and Gower, 1990; Fagioli et al., 1992; Mondadori et al., 1992). To try to understand our present results, we then hypothesized of a greater susceptibility in old flies to peripheral mechanisms (sensory adaptation to sucrose, motor fatigue) which might have increased their suppression performances. Thus we designed Experiment 2 to test this hypothesis. The results however were not so easy to interpret since they showed on the one hand that the proportion of pseudo-habituated flies did not significantly increase with age and on the other hand that once the peripheral effects were removed, the suppression performance no longer differed with age. Such a result in the habituation learning is consistent with the one reported in a previous study (Le Bourg, 1983) where young (7-8 days old) and old (41-42 days old) *Drosophila* were compared in a somewhat different procedure. They are however different from those of a more recent study (Fois et al., 1991) showing that flies were slower to habituate from a middle age.

Finally, as a first attempt in *Drosophila* we did not reveal aging effects in short-term-memory. Future experiments may plan still to use longer ITI to try again to assess the existence of such aging effects. At the same time, the possible influence of various peripheral effects on learning will have to be taken into account. Henceforward, the design of future experiments should include a comparison between experimental and their yoked control groups in order to be aware of the involvement of non associative mechanisms in any conditioning situation.

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